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# Stimulation of Metamorphosis in the Polychaete *Hydroides elegans* Haswell (Serpulidae)

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**Abstract.** The serpulid polychaete *Hydroides elegans* is a common, cosmopolitan warm-water biofouling organism. Competent larvae of *H. elegans* metamorphose rapidly after induction by marine biofilms. Only 15 min after coming in contact with the metamorphic cue, larvae have completed secretion of the primary tube; secretion of the secondary, calcareous tube begins 1.5 h after the primary tube has been deposited. Metamorphosis is characterized by disappearance of the prototroch and differentiation of the tentacular crown in the head region, the collar and thoracic membrane in the thoracic region, and the pygidium at the tip of the abdomen. These morphogenetic events were used to gauge the responses of larvae to biofilms, as well as to the artificial inducers $Cs^+$ and $K^+$. A maximal metamorphic response to the two ions requires exposure to different concentrations and durations, *i.e.*, a 3-h pulse of 10 m*M* CsCl, or a 24-h continuous exposure to 50 m*M* excess KCl. The metamorphic response to $Cs^+$ or $K^+$ is much slower than the response to biofilms, demonstrating that the tissues respond differently to artificial inducers. The differences in the kinetics of the responses to the natural and cationic inducers suggest that the induction mechanisms are not the same. When these artificial inducers were used, some, but not all, of the metamorphosed juveniles never attached to the substratum or secreted a primary tube, probably as a result of secondary effects of the ions on processes of tube formation. The exact mechanisms by which $Cs^+$ and excess $K^+$ induce metamorphosis are still unclear, although we assume, as do others, that these agents act by depolarizing the membranes of excitable sensory cells and not by interacting with specific receptors.

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## Introduction

The influence of ions on invertebrate metamorphosis, first reported by Müller and Buchal (1973) for the larvae of *Hydractinia echinata* more than 2 decades ago, has been demonstrated for a large number of species (summarized in Herrmann, 1995; Woollacott and Hadfield, 1996). Monovalent cations, particularly $K^+$ and $Cs^+$, are clearly established as potent inducers of metamorphosis in several groups, although they are ineffective in some species and actually inhibit metamorphosis in others (Rittschof *et al.*, 1986; Eyster and Pechenik, 1988; Morse *et al.*, 1988; Rowley, 1989). Metamorphosis in response to excess $K^+$ has been reported for seven phyla, and to $Cs^+$ in at least four phyla. How and where these ions act in the metamorphic pathway and how their action is related to that of natural inducers remain unclear.

The most direct approach to metamorphosis-inducing mechanisms of cations and other artificial inducers would be a series of electrophysiological studies of larval sensory organs made during the development of competence. Unfortunately, the small size of most invertebrate larvae makes such studies difficult or impossible. The more usual approach is illustrated by some studies on gastropod larvae. Larvae of the tropical nudibranch *Phestilla sibogae* respond to choline (Hirata and Hadfield, 1986) and excess $K^+$ induction (Yool *et al.*, 1986; Pechenik *et al.*, 1995) only after they are competent to respond to the natural inducer (an extract of coral). In contrast, larvae of the prosobranch gastropod *Crepidula fornicata* do not become responsive to natural cues until 12 to 24 h after they can be induced to metamorphose by excess $K^+$ (Pechenik and Gee, 1993). Differences in the timing of sensitivity to the different inducers has been interpreted as indicative of operation at different sites in an inductive-transduction pathway, but the mechanisms by which vari-

ous artificial inducers act may also develop at different rates.

In the present research, we exposed larvae of *Hydroides elegans* to a natural biofilm and produced a detailed timetable of the resulting events of settlement and metamorphosis. We then compared this developmental timetable with one describing the metamorphic events induced by the monovalent cations $Cs^+$ and $K^+$ when groups of sibling larvae were exposed to the different inducers at the same ages. Because nearly all who have used potassium as an inducer of metamorphosis in invertebrate larvae hypothesize a role for depolarization in induction (*e.g.*, Yool *et al.*, 1986), inductions were also carried out in the presence of a $K^+$-channel blocker. These investigations took advantage of previous work in our laboratory that demonstrated that larvae of *H. elegans* settle preferentially on well-developed marine biofilms, where dominant bacterial species or their extracellular products appear to be the source of a metamorphic cue (Hadfield *et al.*, 1994).

## Materials and Methods

### *Collection of adults and culture of larvae*

Adults of *Hydroides elegans* were collected from Pearl Harbor, Hawaii, and kept at the Kewalo Marine Laboratory of the University of Hawaii in 125 × 46 × 15 cm trays supplied with running seawater. When gametes were needed, several worms were removed from their calcified tubes, whereupon they released eggs or sperm into the seawater, where fertilization occurred. Embryos were cultured at 5–10/ml in glass beakers at room temperature (24°–26°C) in 2 l of natural seawater that had been passed through a 0.22-$\mu$m filter (FSW). After 48 h, when they had achieved the trochophore stage, the larvae were collected on a 41-$\mu$m mesh sieve and transferred to a beaker containing 2 l of freshly filtered seawater. The larvae were fed daily on *Isochrysis galbana* "Tahitian Strain" at a density of about 60,000 cells/ml. The larvae were subsequently transferred to fresh seawater and fed daily until they attained metamorphic competence as three-segmented nectochaetes, usually 4–5 days after fertilization. Competence is defined as the ability of larvae to settle and metamorphose when exposed to a well-developed marine biofilm.

### *Induction of metamorphosis*

Microscope slides were suspended in laboratory tanks supplied with running seawater; after 3 days the slides had accumulated sufficient biofilm to induce metamorphosis of 5-day-old competent larvae. Ten to twenty larvae were added to a drop of seawater on a biofilmed slide, and a supported coverslip was placed over the drop. The seawater beneath the coverslip was replaced periodically by adding fresh seawater with a pasteur pipette at one end of the coverslip and removing the seawater from the other end with absorbent paper. Under these conditions, larvae remained healthy beneath the coverslips for up to 20 h and completed normal metamorphosis. For 14 h after being placed on the biofilmed slides, the larvae were videotaped at 16× magnification through a compound microscope. Images were recorded for 30–60 s approximately every 15 min during the first hour, and about once per hour after that. The series of events from exposure to the biofilm to completion of metamorphosis was characterized by frame-by-frame analysis of the videotape.

### *Assay methods*

Competent larvae aged 4–6 days were used in all experiments except those in which the ontogeny of the metamorphic response was examined; in the latter, testing for induction began 3 days after fertilization. All assays were conducted in 5-ml plastic petri dishes using either FSW or artificial seawater (ASW) (Cavanaugh, 1956). Twenty to sixty competent larvae were pipetted into each dish containing a test solution, and the number of larvae that had metamorphosed was determined 12, 24, 48, or 72 h after the initial induction by observing the larvae through a dissecting microscope. Larvae were considered to have metamorphosed when they had resorbed the prototroch, showed the characteristic differentiation of the body regions that distinguish the juvenile worm and had developed branchial rudiments that indicate early juvenile development.

Five different experiments were conducted to test the effects of $Cs^+$ and excess $K^+$ as artificial inducers.

*Continuous exposure.* Larvae were exposed to the test solution throughout the experimental period. Response to excess $K^+$ was tested in seawater with KCl concentration increased by 10, 20, 30, 50, 60, 80, 100, or 150 m*M*. Effects of $Cs^+$ were tested by adding CsCl to seawater at 2.5, 5, and 10–80 m*M* in 10-m*M* steps.

*Pulse exposure.* Larvae were exposed to the test solution for a determined period and then transferred to seawater free of added ions. In some experiments, larvae were removed from the solution by collecting them on 41-$\mu$m mesh, and in the remainder most of the water was removed from the larval dish with a 5-ml automatic pipettor with a filter across its tip. In both cases the solution was replaced with fresh FSW in which the larvae remained until the end of the experiment. Pulse durations of 20, 40, and 60 min, and 3, 4, 6, 24, and 48 h, were used with 20, 30, and 40 m*M* excess $K^+$, and shorter pulses of 3,

10, 15, 60, and 120 min were employed with 60, 70, and 80 m*M* excess $K^+$. In the cesium experiments, pulse durations were 1, 1.5, 2, 3, 4.5, 6, 7, and 24 h with 10 m*M* CsCl as the metamorphic inducer.

*Interactions among inducers.* Larvae were exposed to a marine biofilm (the natural inducer) in seawater containing 50 m*M* excess KCl for 24 h, or 10 m*M* CsCl for 3 h. After these periods, the biofilm was retained, but the medium was replaced with unaltered seawater. Metamorphic responses were compared with those of larvae induced with a biofilm alone, with excess $K^+$ (50 m*M*) for 24 h, or with $Cs^+$ (10 m*M*) for 3 h alone.

*Effects of the potassium-channel blocker tetraethylammonium chloride (TEA).* Four concentrations of TEA (1, 10, 15, and 30 m*M*) were tested for their effect on metamorphosis in the presence and absence of inducers. When excess $K^+$ or $Cs^+$ was used, TEA remained in the treatment for the same amount of time as the inducer (24 h for excess $K^+$ and 3 h for $Cs^+$), after which solutions were replaced with fresh FSW. When a biofilm was used as the inducer, the larvae remained in the presence of biofilm and TEA during the experiment (12 h).

*Ontogeny of the response to inducers.* To observe the ontogeny of the metamorphic response to ionic inducers, optimum concentrations of the ions, determined as described above, were used. From a single batch of developing larvae, a subset was induced to metamorphose every day beginning 3 days after fertilization. For this experiment, larvae were cultured at an initial density of 5/ml. After each experiment, the density of the larvae remaining in the stock culture was readjusted to maintain 5/ml. An additional experiment was performed to quantify how fast larvae respond to a biofilm as inducer. Larvae (ages 3, 4, and 5 days) were exposed to a biofilm, and the number of larvae that began to secrete a primary tube was determined at 5-min intervals during the first 15 min, at 30-min intervals for the next 3 h, then at 6, 8, 20, and 22 h, and at 24 h when the experiment terminated.

In all experiments, pieces of plastic mesh coated with bacterial biofilm in the laboratory seawater tables were used as a positive control to determine that the larvae were metamorphically competent. A negative control, included to measure spontaneous metamorphosis, consisted of placing larvae in FSW or ASW in clean plastic petri dishes during the experimental period. In experiments with pulse exposures to excess $K^+$ or $Cs^+$, larvae in the negative controls were sieved, or the water was removed, in the same way and at the same time as in the experimental treatments to duplicate the manipulation of the larvae in the experimental treatments.

Stock solutions of 1 *M* KCl, 0.542 *M* CsCl, and 1 *M* TEA (Sigma Chem. Co.) dissolved in FSW or ASW were used to make up test solutions. Each experiment was repeated at least three times, except that the response to biofilms with respect to larval age was tested in only one experiment. Data presented here are those from experiments in which minimum and maximum values were recorded in the negative (FSW or ASW) and positive controls (substratum coated with bacterial biofilm), respectively.

### *Statistical analyses*

Proportional response data were arc-sine transformed, and statistical differences among treatments were tested with one-way ANOVA or Kruskal-Wallis ANOVA of ranks when equal-variance tests failed. Pairwise multiple comparisons were tested using the Student-Newman-Keuls method. All tests were conducted with the aid of SigmaStat software.

## Results

### *Induction and completion of metamorphosis*

When 3-, 4-, and 5-day-old larvae of *Hydroides elegans* were exposed to a natural biofilm, only 5-day-old larvae responded to the metamorphic cue during the first hour. Typically 80% of the 5-day-old larvae settled and began metamorphosis; no metamorphosis was detected when 3-day-old larvae were exposed to a biofilm, and only low percentages (39%) of 4-day-old larvae responded to the inducer after 22 h (Fig. 1).

Five-day-old competent larvae started to crawl on the bottom of the dish within 5 min of being exposed to a biofilm. Each larva secreted a sticky thread from its posterior end, and then immediately began secreting the primary tube. Larvae appeared to secrete the primary tube from most of the trunk surface and to shape it by rolling and moving back and forth within it, and periodically erecting their setae, apparently to push the tube material away from the body. Secretion of the primary tube, completed 10–15 min after the initial response to the biofilm, defines the permanent attachment of the larva. As the primary tube is being secreted, the prototroch is resorbed, the larval body elongates, the collar region becomes apparent due to anterior and posterior constrictions, and the pair of lobes that will form the branchial radioles becomes apparent on the anterior surface of the head (Fig. 2). At this point, metamorphosis is complete.

Secretion of the calcareous tube at the anterior edge of the primary tube begins about 1.7 h after the induction of metamorphosis, and new material is then added to the calcareous tube more or less continuously. As the calcareous tube is being secreted, the branchial radioles grow out from the anterior lobes signaling the initiation of juvenile development. The primary tube is never

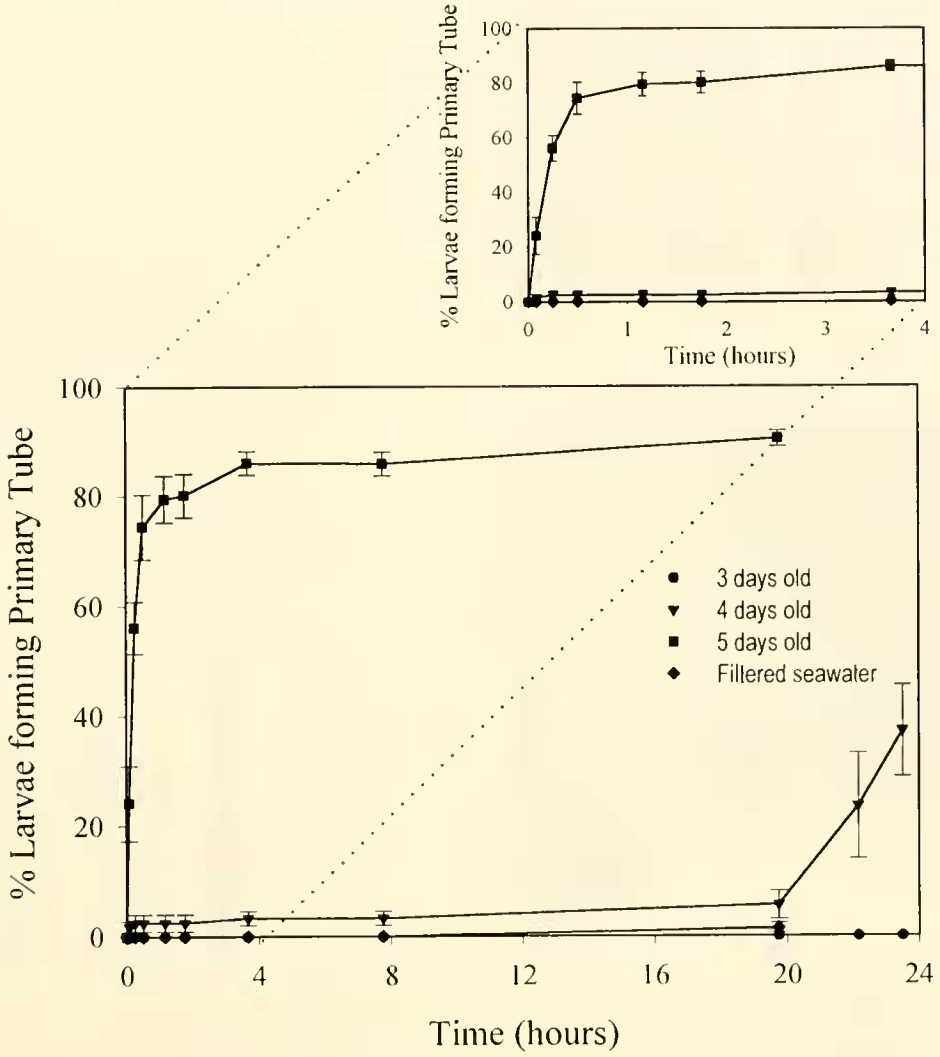


**Figure 1.** Percentage of larvae of *Hydroides elegans* that settled and formed a primary tube after exposure to a marine biofilm. Larvae were 3, 4, or 5 days old at the beginning of the experiment. As a control, 5-day-old larvae were maintained in filtered (22 $\mu$m) seawater without an inducer. Plotted are mean percentages of larvae making a primary tube ± 1 SE ($n$ = 4 replicates/treatment). Insert: detail of the response of larvae during the first 4 h after exposure to a biofilm; symbols are the same as in the main figure.

calcified, and the juvenile has moved completely out of it by 7 h after beginning to metamorphose (Fig. 2).

*Continuous and pulse exposure to metamorphic inducers*

*Effects of excess $K^+$.* Excess $K^+$ in seawater caused competent larvae of *Hydroides elegans* to metamorphose (Fig. 3A). This response was dependent on both duration of exposure and concentration. Up to 77% of competent larvae metamorphosed within 72 h when exposed to 50 m$M$ excess $K^+$ for 24 h. Concentrations higher than 50 m$M$ $K^+$ resulted in fewer metamorphosed larvae, and concentrations higher than 60 m$M$ were toxic. Periods of exposure to excess $K^+$ longer than 24 h resulted in lower frequencies of metamorphosis. Pulse exposure (3, 10, 15, 60, and 120 min) to excess $K^+$ (≥60 m$M$) induced only low percentages of metamorphosis (about 6.5%).

In $K^+$ treatments, some of the metamorphosed larvae (35%–63%) were unable to secrete primary and secondary tubes, even after they were returned to filtered seawater. Despite their tubeless state, some of these worms survived when fed single-celled algae; they grew for up to 2 months, when the cultures were discarded.

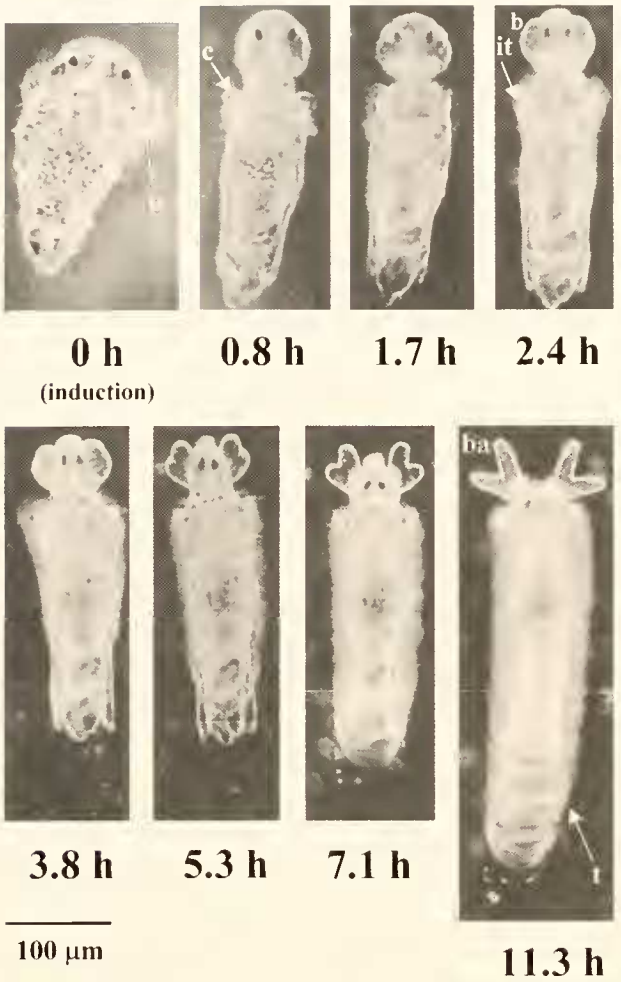


**Figure 2.** Time course of metamorphosis in *Hydroides elegans*. Frames represent a competent larva at the moment of induction to metamorphosis (0 h) and selected stages for the first 11.3 h after induction. p: prototroch, c: collar, b: branchial lobes, it: initiation point of calcareous tube, ba: branchial radioles, t: calcareous tube covering the worm.

*Effects of $Cs^+$.* Larvae of *H. elegans* metamorphosed when exposed to $Cs^+$ in seawater. This response was dependent on concentration and duration of exposure. When the larvae were continuously exposed for 24 h to different concentrations of CsCl, the maximum response occurred with 5 m*M* $Cs^+$ 48 h after induction (24% metamorphosis) (Fig. 3B). Larvae exposed for 24 h to concentrations of CsCl higher than 5 m*M* showed increasing signs of toxicity. In 10 m*M* CsCl, the branchial crown began to form, but never developed further. In the 20-m*M* treatment, the larvae became immobile on the bottoms of the dishes, and at concentrations of 30 m*M* and higher, they died. CsCl applied in pulses of 3–4.5 h produced maximal responses (49%) (Fig. 4A); increases in neither concentration nor length of exposure produced a higher percentage of metamorphosis. At 50 m*M* $Cs^+$, elongated larvae were observed on the bottoms of the dishes; at higher concentrations, the larvae died (Fig. 4B).

*Interactions among inducers*

Metamorphosis in response to a biofilm was greater than 90% in both the presence and absence of excess $K^+$, and these values were significantly higher at 48 h ($P < 0.05$) than those obtained when the larvae were exposed to excess

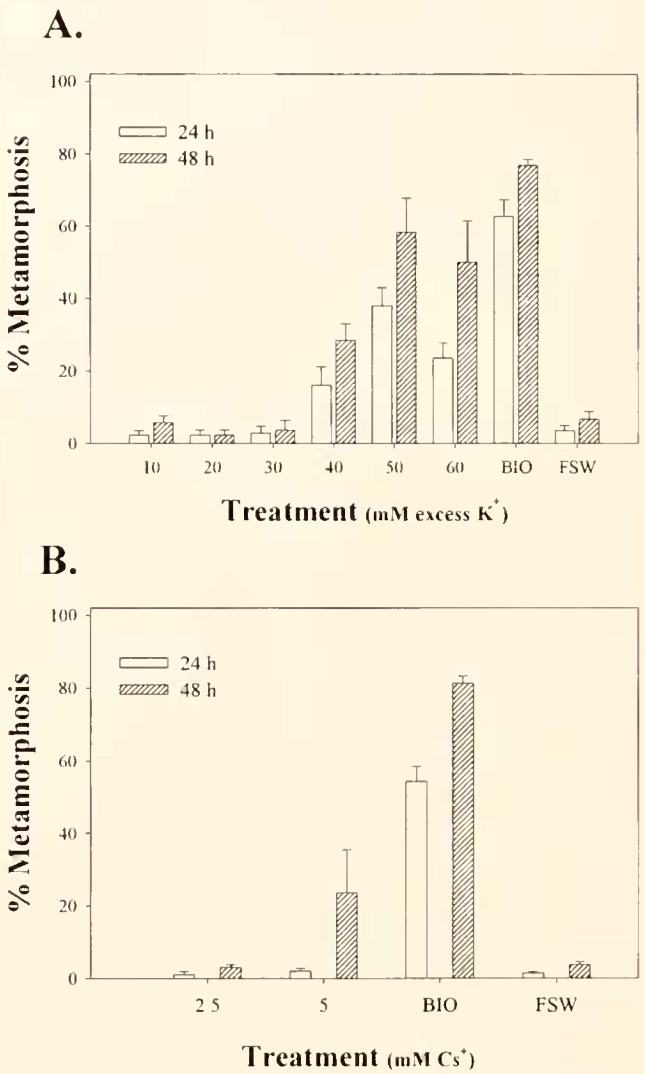


**Figure 3.** Metamorphosis of *Hydroides elegans* exposed to ionic inducers. (A) Exposure to excess $K^+$ in FSW for 24 h; (B) exposure to CsCl in FSW for 24 or 48 h. Bars indicate mean percentages of larvae that metamorphosed ± 1 SE ($n$ = 4 replicates/treatment), evaluated 24 and 48 h after removal of excess $K^+$, or initial exposure to CsCl. BIO = substratum coated with a marine biofilm; FSW = seawater filtered through 0.22-$\mu$m filter.

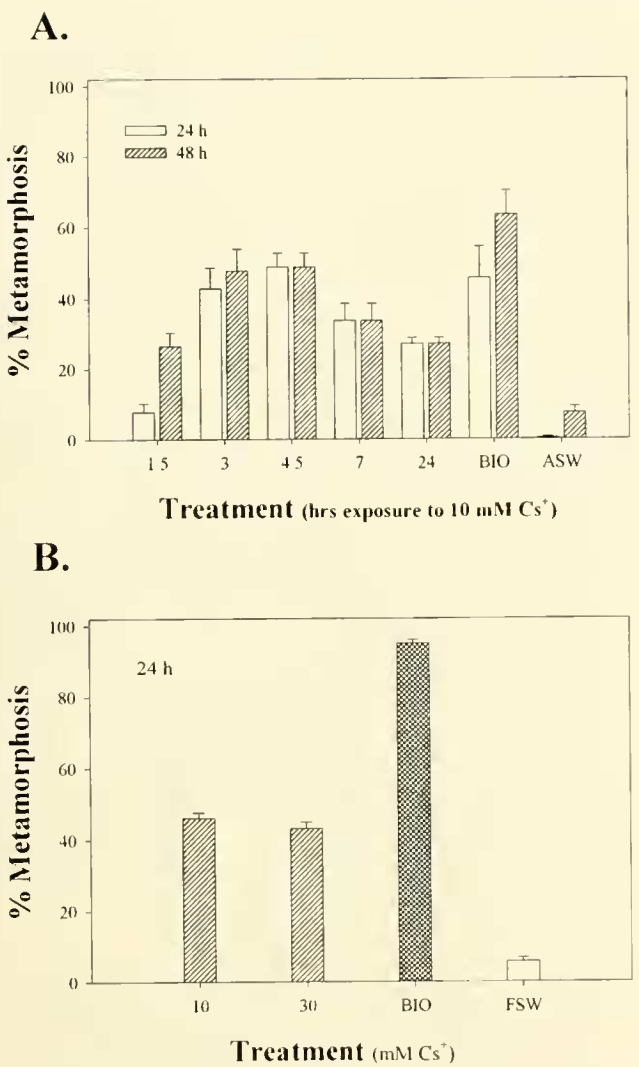


**Figure 4.** Dose-response relationship for cesium applied as a pulse. Metamorphosis of *Hydroides elegans* after exposure to (A) 10 m*M* CsCl for 1.5 to 24 h; (B) 10 or 30 m*M* CsCl for 3 h. Concentrations higher than 30 m*M* were toxic. Bars indicate mean percentages of larvae that metamorphosed ± 1 SE ($n$ = 4 replicates/treatment), evaluated 24 or 48 h after the beginning of the pulse. BIO = substratum coated with a marine biofilm; FSW = seawater filtered through 0.22-$\mu$m filter; ASW = artificial seawater.

$K^+$ alone (66%) (Fig. 5A). When larvae of *H. elegans* were exposed to a biofilm in the presence of $Cs^+$, metamorphosis was higher than with biofilm or $Cs^+$ tested separately (Fig. 5B). These values were significantly different at 24 and 48 h of initial exposure to the inducers ($P < 0.05$). When larvae were induced to metamorphose by a biofilm that had been pre-soaked in seawater containing CsCl, percentages of metamorphosis were similar to those observed with biofilm not exposed to $Cs^+$, demonstrating that cesium ion has no direct effect on the biofilm.

*Effects of tetraethylammonium chloride (TEA)*

TEA inhibited metamorphosis in a concentration-dependent manner when larvae were induced with excess $K^+$ or $Cs^+$. Significant inhibition of metamorphosis ($P < 0.05$) was found when 10 m*M* TEA was tested in the presence of excess $K^+$ for 24 h (Fig. 6A), and when

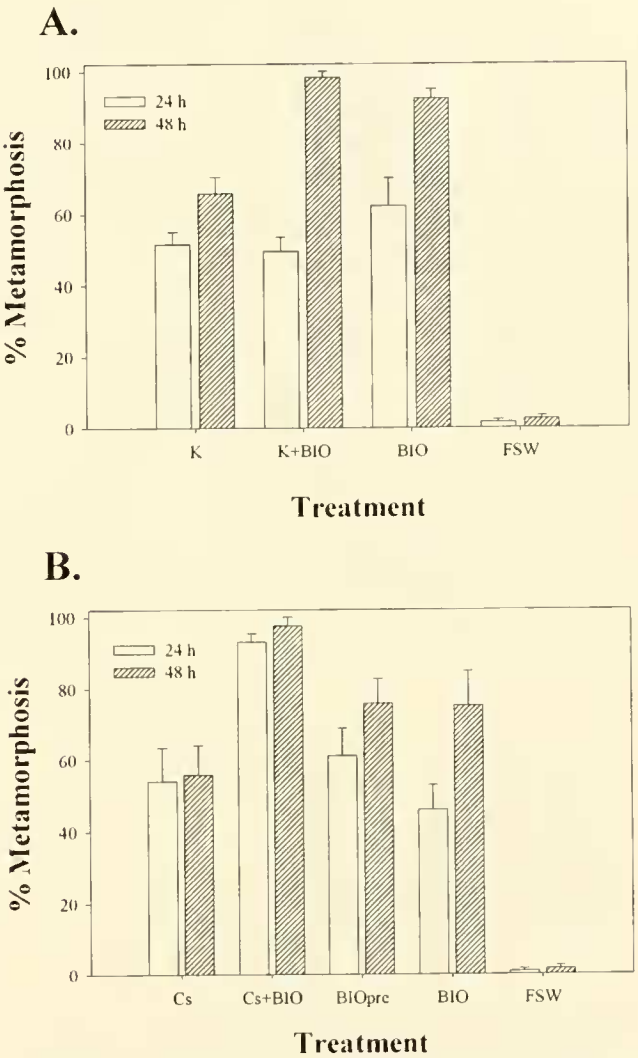


**Figure 5.** Synergism in the effects of ionic inducers and biofilm on metamorphosis of *Hydroides elegans*. (A) Metamorphosis of larvae exposed to 50 m*M* excess $K^+$ for 24 h in the presence or absence of a marine biofilm. (B) Metamorphosis of larvae exposed to a 3-h pulse of 10 m*M* CsCl in seawater in the presence or absence of a marine biofilm. Bars indicate mean percentages of larvae that metamorphosed ± 1 SE ($n$ = 4 replicates/treatment), evaluated 24 and 48 h after initial contact with the inducers. K = 50 m*M* excess $K^+$ for 24 h; Cs = 3-h pulse of 10 m*M* CsCl; BIO = substratum coated with a marine biofilm; FSW = seawater filtered through 0.22-$\mu$m filter; BIOpre = substratum coated with a marine biofilm, soaked in 10 m*M* CsCl for 3 h and returned to FSW before addition of larvae.

30 m*M* TEA was tested in combination with a 3-h pulse of $Cs^+$ (Fig. 6B). Metamorphosis was reduced, but to a lesser extent, when larvae were exposed simultaneously to a biofilm and to 10 or 15 m*M* TEA ($P < 0.05$) (Fig. 6C). At the doses tested, 30 m*M* TEA was toxic after 24 h when tested with an organic biofilm or excess $K^+$.

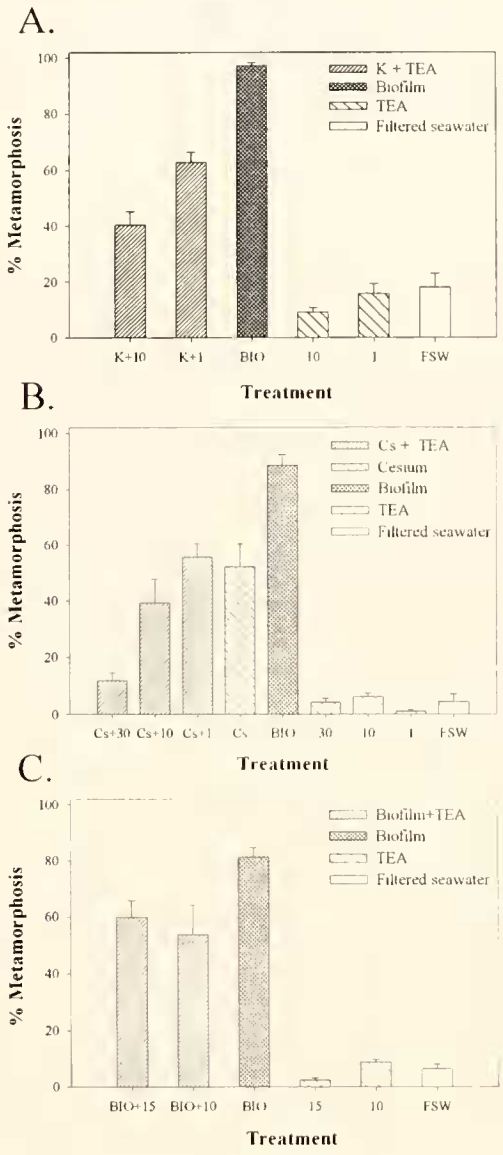


**Figure 6.** Inhibition of metamorphosis of *Hydroides elegans* by the ion channel blocker tetraethylammonium chloride (TEA). (A) Effect of TEA on metamorphosis in the presence or absence of 50 m*M* excess $K^+$ in seawater for 24 h. (B) Effect of TEA on metamorphosis in the presence or absence of a 3-h pulse of 10 m*M* CsCl in seawater. (C) Effect of TEA in the presence or absence of a marine biofilm for 12 h. Bars indicate mean percentages of larvae that metamorphosed ± 1 SE ($n$ = 4 replicates/treatment), evaluated 48 h after initial exposure to excess $K^+$, 24 h after initial exposure to CsCl, and 12 h after initial exposure to a marine biofilm. K = 50 m*M* excess $K^+$ for 24 h; Cs = 3-h pulse of 10 m*M* CsCl; 1, 10, 15 and 30 = m*M* concentrations of TEA; BIO = substratum coated with a marine biofilm; FSW = seawater filtered through 0.22-$\mu$m filter.

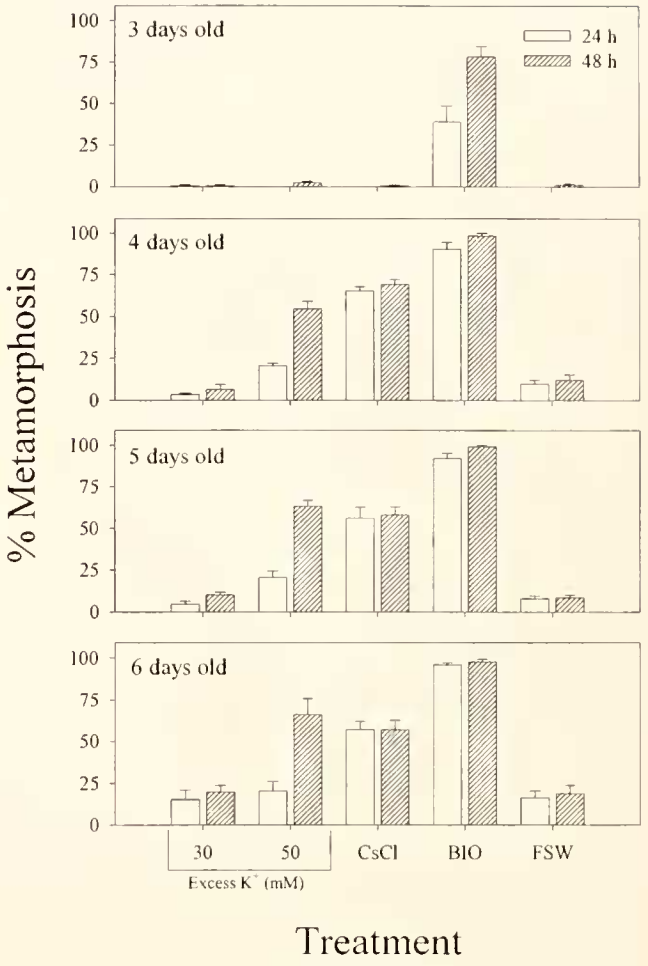


**Figure 7.** Metamorphosis of 3- to 6-day-old larvae of *Hydroides elegans* from a single batch when exposed to different inducers. Larvae were removed from the 10 m*M* CsCl after 3 h and from 30 and 50 m*M* excess $K^+$ after 24 h. Bars indicate mean percentages of larvae that metamorphosed ± 1 SE ($n$ = 4 replicates/treatment). BIO = substratum coated with a marine biofilm; FSW = seawater filtered through 0.22-$\mu$m filter.

*Ontogeny of the metamorphic response to biofilm, $Cs^+$, and excess $K^+$*

The metamorphic responses of larvae, as a function of age (beginning at day 3) and inducing agent, were compared. The earliest response was to biofilm, where 39% of larvae metamorphosed after 24 h of exposure, *i.e.*, at an age of 4 days. In contrast, only negligible numbers of larvae exposed to either $Cs^+$ or $K^+$ on day 3 metamorphosed, even after 48 h (Fig. 7); indeed the first responses to $Cs^+$ and excess $K^+$ were recorded only 24 h after an

initial exposure on day 4. The delayed response to ions is more striking when maximal values are compared. When metamorphosis was induced with excess $K^+$, maximal values were reached 48 h after induction, much later than with a biofilm or $Cs^+$. With all three inducers, maximal values of metamorphosis were obtained with larvae 4 days old or older, and the percentage of metamorphosis was always higher in response to a biofilm than to $Cs^+$ or excess $K^+$. Larvae remained responsive to the inducers through the last day of the experiment, when they were 6 days old.

## Discussion

### *Morphogenesis during metamorphosis*

When larvae of *Hydroides elegans* are exposed to a well-developed marine biofilm, they respond rapidly by exploring the surface of the substratum for 1–2 min, attaching, secreting a primary tube, and beginning to metamorphose, all within 1–15 min of initial exposure (Fig. 1). The main morphogenetic events of metamorphosis are completed within 11–12 h of initial contact with a biofilm (Fig. 2). These developmental events progress more slowly when metamorphosis is induced with ions (excess $K^+$ and $Cs^+$). Wisely (1958), in an earlier report on metamorphosis in this species, described secretion of an "adhesive substance," but did not mention the behavioral phase when larvae appear to examine the substratum.

In *H. elegans,* secretion of the primary tube and simultaneous elongation of the larva and resorption of the prototroch cilia are irreversible steps. The subsequent differentiation of the tentacular crown defines early juvenile development.

The observation that competent larvae of *H. elegans* respond to bacterial biofilms within 15 min, together with evidence presented by Hadfield *et al.* (1994) that settlement of *H. elegans* increases linearly with bacterial cell count, demonstrates that bacteria are an important source of stimulus for settlement and metamorphosis in this species. In addition, Walters *et al.* (1997) found that larvae of *H. elegans* do not settle preferentially on or near conspecific individuals, living or dead, in the field or in laboratory dishes, and that natural settlement patterns are explained by hydrodynamic processes and the presence of biofilms. We thus suggest that the latent response of larvae of *H. elegans* to extracts of adult worms described by Bryan *et al.* (1997) probably results from the buildup of bacteria in their test vessels. The gregarious response of larvae of *Hydroides dianthus* described by Scheltema *et al.* (1981) and Toonen and Pawlik (1996) is apparently a character not shared by all species in this genus.

The data provided here on induction of metamorphosis of *H. elegans* with excess $K^+$ or $Cs^+$ expand on a growing literature on this subject (see summaries in Herrmann, 1995; Woollacott and Hadfield, 1996). *Hydroides elegans* is not the first polychaete to be found sensitive to potassium or cesium (Herrmann, 1995). Yool *et al.* (1986) were able to induce maximum percentages of metamorphosis in larvae of *Phragmatopoma californica* at lower potassium concentrations (5–20 m*M*) than those found to be optimal for *H. elegans* (50 m*M*). In addition, percentages of metamorphosis were lower in *H. elegans* (77% *versus* 100% for *P. californica*). Differences in $K^+$ sensitivity between species could explain these results: species-specific metamorphic responses to excess $K^+$ were recently reported by Pearce and Scheibling (1994) for the echinoids *Strongylocentrotus droebachiensis* and *Echinarachnius parma.*

The metamorphic response of larvae of *H. elegans* to excess $K^+$ was slower than to $Cs^+$ or a biofilm. The larvae took 72 h to achieve maximum levels of metamorphosis in response to $K^+$, and metamorphosis was typically completed 24 h or longer after removing them from seawater with excess $K^+$. A similar delay in the metamorphic response was found in *Phestilla sibogae,* when induction was compared between the natural inducer (a coral metabolite) and choline (Hirata and Hadfield, 1986) or excess $K^+$ (Pechenik *et al.,* 1995). In contrast, larvae of the prosobranch *Crepidula fornicata* become sensitive to induction by excess $K^+$ 12–24 h before they are responsive to natural inducers (Pechenik and Gee, 1993). These differences may arise from nothing more complex than the degree of exposure of sensory cells to the external medium or the resistance of sensory cells to depolarization by external ion shifts—in other words, to plasticity in induction mechanisms at the species level, as suggested by Woollacott and Hadfield (1996).

It has been proposed that excess $K^+$ induces metamorphosis by depolarization of externally accessible excitable cells (Baloun and Morse, 1984; Yool *et al.,* 1986). However, the possibility remains that $K^+$ acts on the entire nervous system, producing a generalized activation (Todd *et al.,* 1991), or that the ions exert a direct effect on target tissues (Yool *et al.,* 1986).

The observation that most larvae of *H. elegans* metamorphose without tube formation when induced by potassium ions may indicate that at least part of the action of this ion is directly on target tissues. However, it is more likely that the altered ionic makeup of the seawater disrupts secretion of the primary-tube proteins (possibly by inhibiting proper cross-linking) by epidermal cells. The lengthy exposure to excess $K^+$ required to induce maximum metamorphosis concomitantly leads to large numbers of tubeless metamorphosed worms. Although failure to produce a primary tube prevents a larva from attaching to hard substrata, the morphogenetic events of metamorphosis are completed. In other words, metamorphic

morphogenesis is not dependent on secretion of the primary tube. Potassium ions produced an analogously disturbed metamorphosis in the brachiopod *Terebratalia transversa;* many of the larvae underwent partial metamorphosis, characterized by protegulum (or initial shell) formation without settlement (Freeman, 1993). Clearly, care must be used in ascribing singular modes of action to events elicited when an entire sensitive larva is immersed in seawater with an altered ion composition.

Larvae of *H. elegans* metamorphosed faster in response to $Cs^+$ than to excess $K^+$. These results are difficult to interpret because of the required short-pulse exposure necessary with cesium compared to the 24-h exposure required for maximum response to potassium. That the larvae first become sensitive to these ions at about the same age suggests either a similar inductive mechanism or a common site of action in the metamorphic pathway; reasoning presented below supports the latter hypothesis.

The one or more mechanisms by which $Cs^+$ induces metamorphosis in marine invertebrate larvae have not been determined with certainty, although $Cs^+$ has been tested with positive results in coelenterates (Freeman and Ridgway, 1987; Hujer and Lesh-Laurie, 1995), phoronids (Herrmann, 1995), a sponge (Woollacott and Hadfield, 1996), and a polychaete (Herrmann, 1986). Cesium is a recognized potassium-channel blocker and, when externally applied, leads to depolarization of the plasma membrane in some systems (Hille, 1992). This depolarization can activate voltage-dependent calcium channels, releasing calcium ions into the cytoplasm and modulating a wide variety of cell processes, including secretion, contraction, and the cell cycle (Clapham, 1995). Cesium activation of calcium-mediated mechanisms in invertebrate larvae has been documented mainly in hydrozoans. Leitz and Müller (1987) found a fast increase of inositol trisphosphate ($IP_3$) concentration after incubation of planulae of *Hydractinia echinata* in 116 m*M* CsCl. $IP_3$ is known to induce release of $Ca^{2+}$ from internal stores by activating receptors on the endoplasmic reticulum. Freeman and Ridgway (1990) observed that calcium transients were produced when larvae of the hydrozoan *Mitrocomella polydiademata* were induced to metamorphose by $Cs^+$. Ilan *et al.* (1993) demonstrated a role for intracellular calcium in regulating metamorphosis in the polychaete *Phragmatopoma californica;* larvae metamorphosed in response to elevated external $Ca^{2+}$ concentrations and to calcium ionophores. A role for calcium during metamorphosis of *H. elegans* appears certain despite the fact that our preliminary experiments with this species produced results that were different from those reported by Ilan *et al.* (1993) for *P. californica.* In *H. elegans,* metamorphosis was inhibited when induced in the presence of calcium ionophore A23187 or one of the calcium-channel blockers nefedipine and verapamil. These results and the effects of TEA, discussed below, suggest that the mechanism of action of $Cs^+$ on larvae of *H. elegans* may be different from the way it acts on planula larvae and, perhaps, on larvae of *P. californica.* Experiments are under way in our laboratory to clarify the role of $Ca^{2+}$ in the metamorphosis of *H. elegans.*

If, as is generally assumed, the natural inducer (a marine biofilm) and potassium ions both lead to metamorphosis by initiating membrane depolarization and generation of a receptor potential in excitable, external sensory cells, the inhibition of such induction by TEA, a potassium-channel blocker, is understandable. TEA, by blocking $K^+$ channels, inhibits ion flow and membrane depolarization. The observation that TEA also inhibits cesium-induced metamorphosis appears contradictory at first, because $Cs^+$ is itself a potassium-channel blocker in some organisms (Hille, 1992). However, potential explanations are at hand. The concentration of cesium found to induce metamorphosis in *H. elegans* (10 m*M*) is much lower than the concentration of potassium ion found necessary to induce metamorphosis (50 m*M* in excess of that normally present in seawater) and is thus unlikely to act by simply depolarizing the membrane of a receptor cell. It is more likely that $Cs^+$ enters receptor cells through potassium channels, as has been shown to occur in *Drosophila* potassium channels expressed in *Xenopus* oocytes (Heginbotham and MacKinnon, 1993), and acts inside the cells. TEA blockage of $K^+$ channels in larvae of *H. elegans* would thus directly inhibit cesium inflow and metamorphic induction. A possible target for $Cs^+$ inside the cells may be the ATP-driven $Na^+/K^+$ pump, whose major function is maintenance of the transmembrane gradient and thus the resting membrane potential (Petersen, 1992).

When competent larvae were exposed to a biofilm in the presence of $Cs^+$ or excess $K^+$, a synergistic effect was noted (24 h after removal from excess $K^+$). This synergism may be understood as a partial depolarization of excitable cells that reduces the threshold necessary to activate metamorphosis by the natural biofilm inducer; this mechanism was suggested by Woollacott and Hadfield (1996) as a possible explanation for the interaction of ions and biofilm in the induction of metamorphosis of larvae of a sponge. The more apparent synergism noted between $Cs^+$ and a biofilm probably arises from the more rapid (compared to potassium) action of cesium as an inducer. Given the 24-h exposure necessary to achieve maximum metamorphosis with potassium, most larvae will already have metamorphosed from continued contact with the biofilm.

Larvae must be exposed to excess potassium longer than to biofilms to achieve metamorphosis, and it is therefore impossible to distinguish between the possibilities

(1) that larval sensitivity to $K^+$ develops later than sensitivity to biofilms and (2) that the larval response to $K^+$ simply takes longer. The response to a biofilm that can be seen after a 24-h exposure in 3-day-old larvae does not occur when potassium (or cesium) is used as an inducer (Fig. 7). However, it may be that the larvae that respond to a biofilm when exposed at day 3 became competent only in the last moments of exposure; that is, when they were almost 4 days old. Because the larvae tested with potassium were removed from the inducer at this time, they were deprived of the necessary 24-h exposure after becoming competent, and thus never metamorphosed at all. The argument is similar for cesium. Day 3 larvae were exposed to cesium for only 3 h, and thus were not in the inducer when they became metamorphically competent (Fig. 7). The result is that no larvae metamorphosed after 24 or 48 h. Differences in the age at onset of sensitivity to biofilms and the ions cannot be discerned from our experimental results.

Despite possible variation in the age at which larvae of *Hydroides elegans* become sensitive to different inducers, the hypothesis that metamorphic induction is primarily a chemoreceptive process, including depolarization of an excitable receptor cell and transmission of the metamorphic signal via the nervous system, is still the most parsimonious. Moreover, the ability of larvae of *H. elegans* to metamorphose without primary tube formation as a response to potassium or cesium demonstrates that steps in the settlement process may be separated, with some processes proceeding while others do not. Our current work includes exploration of the transduction pathway associated with metamorphic induction. We seek to understand the mechanisms by which the chemoreceptive act of induction is transduced into a cellular signal as well as the manner in which the metamorphic stimulus activates new gene transcription or translation.

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